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(54) Title: PHTHALOCYANINE AND PORPHYRAZINE PHARMACEUTICAL COMPOSITIONS

(57) Abstract: Pharmaceutical compositions containing a neutral or negatively charged compound having a phthalocyanine structure or one of the porphyrazines or the metal-complex formed thereof are effective in decreasing infection by HIV and other pathogens leading to sexually transmitted diseases. The compositions can be made suitable for any mode of administration. Preferably, the composition is suitable for topical administration, especially for mucosal administration. The most preferred composition is suitable for vaginal or rectal administration.

PHTHALOCYANINE AND PORPHYRAZINE
PHARMACEUTICAL COMPOSITIONS

Cross Reference to Related Applications

Priority is claimed to U.S. Provisional application Serial No. 60/349,944, filed January 18, 2002.

Statement Regarding Federally Funded Research

The Federal Government has certain rights in the invention disclosed herein by virtue of Grant No. AI45883 to R.W. Compans from the National Institute of Health.

Background Of The Invention

This application relates to the field of anti-viral compounds, specifically phthalocyanine compounds, for the prevention of sexually transmitted diseases (STDs) caused by viral pathogens such as human immunodeficiency virus, herpes viruses, hepatitis viruses, and papilloma viruses.

Sexually transmitted diseases (STDs), once called venereal diseases, are among the most common infectious diseases in the United States today. More than 20 STDs have now been identified, and they affect more than 13 million men and women in this country each year. The annual comprehensive cost of STDs in the United States is estimated to be well in excess of $10 billion.

STDs affect men and women of all backgrounds and economic levels. They are most prevalent among teenagers and young adults. Nearly two-thirds of all STDs occur in people younger than 25 years of age. The incidence of STDs is rising, in part because in the last few decades, young people have become sexually active earlier yet are marrying later. In addition, divorce is more common. The net result is that sexually active people today are more likely to have multiple sex partners during their lives and are potentially at risk for developing STDs.

Health problems caused by STDs tend to be more severe and more frequent for women than for men, in part because the frequency of asymptomatic infection means that many women do not seek care until

serious problems have developed. Some STDs can spread into the uterus (womb) and fallopian tubes to cause pelvic inflammatory disease (PID), which in turn is a major cause of both infertility and ectopic (tubal) pregnancy. The latter can be fatal. STDs in women also may be associated with cervical cancer. One STD, human papillomavirus infection (HPV), causes genital warts and cervical and other genital cancers. STDs can be passed from a mother to her baby before, during, or immediately after birth; some of these infections of the newborn can be cured easily, but others may cause a baby to be permanently disabled or even die.

HIV Infection and AIDS

AIDS (acquired immunodeficiency syndrome) was first reported in the United States in 1981. It is caused by the human immunodeficiency virus (HIV), a virus that destroys the body's ability to fight off infection. An estimated 900,000 people in the United States are currently infected with HIV. People who have AIDS are very susceptible to many life-threatening diseases, called opportunistic infections, and to certain forms of cancer. Transmission of the virus primarily occurs during sexual activity and by sharing needles used to inject intravenous drugs.

Genital Herpes

Genital herpes affects an estimated 60 million Americans. Approximately 500,000 new cases of this incurable viral infection develop annually. Herpes infections are caused by herpes simplex virus (HSV). The major symptoms of herpes infection are painful blisters or open sores in the genital area. These may be preceded by a tingling or burning sensation in the legs, buttocks, or genital region. The herpes sores usually disappear within two to three weeks, but the virus remains in the body for life and the lesions may recur from time to time. Severe or frequently recurrent genital herpes is treated with one of several antiviral drugs that are available by prescription. These drugs help control the symptoms but do not eliminate the herpes virus from the body. Suppressive antiviral therapy can be used to prevent occurrences and perhaps transmission. Women who acquire genital herpes during pregnancy can transmit the virus to their babies. Untreated HSV infection in newborns can result in mental retardation and death.

Genital Warts

Genital warts (also called venereal warts or condylomata acuminata) are caused by human papillomavirus, a virus related to the virus that causes common skin warts. Genital warts usually first appear as small, hard painless

5 bumps in the vaginal area, on the penis, or around the anus. If untreated, they may grow and develop a fleshy, cauliflower-like appearance. Genital warts infect an estimated 1 million Americans each year. In addition to genital warts, certain high-risk types of HPV cause cervical cancer and other genital cancers. Genital warts are treated with a topical drug (applied to the skin), by

10 freezing, or if they recur, with injections of a type of interferon. If the warts are very large, they can be removed by surgery.

Other diseases that may be sexually transmitted include chlamydial infection, gonorrhea, syphilis, trichomoniasis, bacterial vaginosis, cytomegalovirus infections, scabies, and pubic lice. STDs in pregnant

15 women are associated with a number of adverse outcomes, including spontaneous abortion and infection in the newborn. Low birth weight and prematurity appear to be associated with STDs, including chlamydial infection and trichomoniasis. Congenital or perinatal infection (infection that occurs around the time of birth) occurs in 30 to 70 percent of infants born to

20 infected mothers, and complications may include pneumonia, eye infections, and permanent neurologic damage.

This starts on AIDS again– do you want header here?

AIDS, or acquired immunodeficiency disease, is characterized by an imbalance in two basic types of immune system cells, helper/inducer T

25 lymphocytes and suppressor T lymphocytes, with the ratio of suppressor cells to helper/inducer cells greatly elevated. Helper/inducer T cells, defined by a surface antigen called CD4, are responsible for the induction of most of the functions of the human immune system, including the humoral immune response involving the production of antibodies by B lymphocytes and the

30 cell-mediated response involving stimulation of cytotoxic T cells. A condition associated with HIV is AIDS-related complex, or ARC. Most human beings suffering from ARC eventually develop AIDS.

Two related retroviruses can cause AIDS, human immunodeficiency

virus type 1 and type 2 (HIV-1 and HIV-2, generally referred to herein as

HIV). The genomes of the two viruses are about 50% homologous at the

nucleotide level, contain the same complement of genes, and appear to attack

5   and kill the same human cells by the same mechanism. Also known as LAV

(lymphadenopathy-associated virus), HTLV-3 (human T-lymphotropic virus-

type 3), and ARV (AIDS-related virus), HIV-1 was identified in 1983.

Virtually all AIDS cases in the U.S. are associated with HIV-1 infection.

HIV-2 was isolated in 1986 from West African AIDS patients.

10   Both types of HIV are retroviruses, in which the genetic material is

RNA rather than DNA. The viruses carry with them a polymerase (reverse

transcriptase) that catalyzes transcription of viral RNA into double-helical

DNA. The viral DNA can exist as an unintegrated form in the infected cell or

be integrated into the genome of the host cell. As presently understood, the

15   HIV enters the T4 lymphocyte where it loses its outer envelope, releasing

viral RNA and reverse transcriptase. The reverse transcriptase catalyzes

synthesis of a complementary DNA strand from the viral RNA template. The

DNA helix then inserts into the host genome where it is known as the

provirus. The integrated DNA may persist as a latent infection characterized

20   by little or no production of virus or helper/inducer cell death for an

indefinite period of time. When it is transcribed by the infected lymphocyte,

new viral RNA and proteins are produced to form new viruses that bud from

the cell membrane and infect other cells.

No prevention capable of preventing or reversing the

25   immunodeficiency of AIDS or ARC is currently available. All patients with

opportunistic infections and approximately half of all patients with Kaposi's

sarcoma die within two years of diagnosis. Attempts at reviving the immune

systems in patients with AIDS have been unsuccessful.

A number of compounds have apparent virucidal activity against this

30   virus, including HPA-23, interferons, ribavirin, phosphonoformate,

ansamycin, suramin, imuthiol, penicillamine, carbovir, 3'-azido-3'-

deoxythymidine (AZT), and other 2',3'-dideoxynucleosides, such as 2',3'-

dideoxycytidine (DDC), 2',3'-dideoxyadenosine (DDA), 2',3'-dideoxyinosine

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(DDI), 3'-azido-2',3'-dideoxyuridine (CS-87), 2',3'-dideoxy-2',3'-didehydrocytidine (D4C), 3'-deoxy-2',3'-didehydrothymidine (D4T) and 3'-azido-5-ethyl-2',3'-dideoxyuridine (CS-85). However, all are administered systemically, are expensive, and have serious side effects. The virus also

5   readily mutates to yield drug resistant.

Inhibitors of cellular processes will often limit viral replication. Unfortunately, they are also usually toxic for the host and therefore cannot be prescribed for a prolonged period of time because of their toxicity. Efforts to decrease the problem of toxicity have primarily been directed towards

10   finding selective, less toxic drugs. Due to the exorbitant cost of the nucleoside type drugs, research has also been centered around compounds which are relatively easy and economical to manufacture.

Herpes Simplex

Herpes simplex viruses, particularly herpes simplex virus type 2

15   (HSV-2), are common sexually transmitted pathogens. Following transmission of the virus to a susceptible individual, HSV-2 replicates in the epithelial cells of genital mucosal surfaces. This replication is usually asymptomatic, as evidenced by the number of individuals who are seropositive for HSV-2 antibody, but have no history of symptomatic

20   infection. However, particularly in individuals who are seronegative for both HSV-1 and HSV-2, primary infection can result in severe, ulcerative lesions. Following replication in epithelia, the virus infects the peripheral endings of sensory neurons innervating the site of infection, and is transported through the neuronal axons to the nuclei.. Viral DNA enters the neuronal nuclei and

25   latent infections are established. Various stimuli, including stress, damage to peripheral tissues near the site of infection, or direct nerve damage cause reactivation of latent virus, and productive viral replication is initiated in the neuron.. Virus is transported back through neuronal axons to the epithelial tissue, where it again replicates, is shed into extracellular space, and is

30   available for transmission to a new individual (Whitley, et al. *Ann Intern Med.* 125(5):376-83 (1996); Roizman and Sears, Annu. Rev. Micrbiol. 41:543-571 (1987)).

Because the latent infection lasts for the lifetime of the host, infection by HSV has the potential to result in many episodes of recurrent disease and transmission. As with the initial infection, many of these recurrent infections are asymptomatic, so that neither the infected individual or his or her partner

5   may be aware of the risk of transmission. Regular use of virucidal compounds by women who believe they are uninfected would reduce not only their own risk of infection, but would reduce the risk of transmission to new partners of women with asymptomatic recurrences.

In addition to genital infection, HSV-2 is the most common cause of

10   neonatal herpes infection, which is most frequently transmitted during delivery of an infant to a mother who is shedding infectious virus (Whitley, 1996). Availability of nontoxic, topical virucidal compounds, and their use during delivery, would reduce or eliminate virus available for transmission and thereby also reduce the level of risk to the infant.

15   Genital herpes infections have also been implicated in the transmission of human immunodeficiency viruses. Epidemiologic studies have suggested that infection by HSV-2, along with other sexually transmitted diseases that cause genital ulcers, increases the risk of acquisition of HIV. The mechanism of this increased risk in unknown, but it may be due

20   to the increased numbers of HIV-susceptible cells (CD4+ T cells and macrophages) present in genital epithelium during the inflammatory immune response generated by the STDs (Latif et al., AIDS. 3:519-523 (1989)). In addition, co-infection of HSV-2 and HIV may result in a higher risk of transmission of HIV: HIV virions have been detected in cells present in

25   genital lesions caused by HSV, leading to the hypothesis that HSV lesions may generated a higher level of HIV in the genital tract available for transmission (Schacker et al. J. Infect. Dis. 178(6):1616-22 (1998); JAMA. 1998 Jul 1;280(1):61-6 (1998)).

There are a number of virucidal drugs available for inactivation of

30   HSV replication, including acyclovir, cidofivir, sorivudine, and foscarnet. However, all of these drugs target replication of the viral DNA following infection of susceptible cells; they cannot prevent the initial infection of epithelial cells. In animal models, several of the drugs have been shown to be

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only partially effective at reducing viral replication in genital epithelium when applied topically (see, for example, Bravo, et. al., Antiviral Res 21:59-72 (1993)). Reduction of epithelial replication during initial infection has been demonstrated in animal models to reduce the amount of latent virus

5   present in ganglia and to reduce the frequency and severity of recurrent disease (Roizman and Sears, 1987). However, other studies have demonstrated that epithelial replication is not a prerequisite for the establishment of latent infection in animal models (Sedarati et al., Virology 192:687-691 (1993)). In addition, the high percentage of women with latent

10   virus but no history of symptomatic infection suggests that in humans, high levels of replication may not be necessary for the establishment of latency. In the absence of an effective vaccine, use of topical virucidal agents may then be the best chance for reducing the number of individuals with latent HSV-2 infections.

15   Anti-HSV virucides tested to date include compounds with both specific and nonspecific activity. Many of these compounds are effective virucides when tested in cell culture, including those that inhibit specific interactions between the virus and the cell surface (neutralizing antibodies and polyanionic compounds such as heparan sulfate, heparin, dextran sulfate,

20   and carageenan), and those that disrupt virion architecture (nonoxynol-9) (see, i.e., Zeitlin et al., Contraception 56: 329-335 (1997); Zacharopoulos and Phillips, *Clinical and Diagnostic Laboratory Immunology* 4:465–468 (1997)). However, use of neutralizing antibodies is cost prohibitive, at least at the present time. Polyanionic compounds have had varying success in

25   inactivation of HSV-2 infection *in vivo*; in a mouse model of genital infection, heparan sulfate was not particularly effective, and dextran sulfate and carageenan prevented infection only of extremely low doses of virus (10^3 pfu or less) (Zeitlin et al. 1997). Continual use of nonoxynol-9 has been shown to cause inflammation of vaginal and cervical epithelium (Stafford, et

30   al., J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. 17, 327-331 (1998)), and to inhibit growth of normal vaginal flora (lactobacilli) that protect the vaginal tract from infection by other pathogens (Stafford et al. 1998). Results of studies to determine the effects of N9 use on transmission of

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STDs, particularly HIV, have varied (Weir et al., Genitourin Med, 71:78-81 (1995)), but it seems far from an ideal topical microbicide for frequent vaginal application.

It is well documented that an active STD contributes to the increase in HIV transmission (Cohen, Science 279:1854-1855 (1998)). Successful prevention of STDs reduces genital shedding of HIV, thus lowering the HIV transmission rate (Cohen, 1998). Although two of the most common STDs, gonorrhea and chacroid, can be treated successfully, the development of antibiotic resistance may seriously compromise efforts to control these STDs. For example, high level of resistance to penicillin and tetracycline in *H. ducreyi* and *N. gonorrhoeae* has been recognized since 1976 (Ison et al., Antimicrobial Agents and Chemotherapy 42:2919-2922 (1998)). The percentage of resistant isolates in the New World to either penicillin or tetracycline approached 40% in 1995 (Ison et al., 1998). Although vaccine development against common STDs has a high priority and has been stimulated by the genome approaches, effective and safe vaccine against gonorrhea, syphilis and chlamydia are not available. Therefore, there is a need for a drug for the prevention of STDs in combination with a drug for the inactivation of HIVs.

It is therefore an object of the present invention to provide compounds having selective virucidal activity against human immunodeficiency virus.

It is therefore another object of the present invention to provide compounds which are effective for the prevention of sexually transmitted viral diseases.
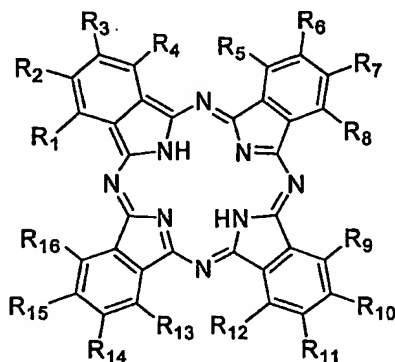
It is a further object of the present invention to provide compounds having virucidal activity for topical administration.

It is a still further object of the present invention to provide compounds having virucidal activity for mucosal administration.
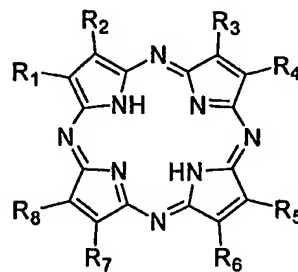
Summary Of The Invention

A method for preventing a sexually transmitted disease (STD) or viral infection with a virus such as HIV in a human by administering to a mucosal surface, preferably prior to infection, a composition containing an

amount of a neutral or negatively charged compound, a metal complex of

the compound, a pharmaceutically acceptable salt of the compound or metal

complex, or a mixture thereof to a human in an amount effective to decrease

a viral infection, wherein the compound has one of the following structures:

5   Formula I   Formula II

wherein $R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}$ and R^{16}

taken independently or together can be hydrogen, alkyl, substituted alkyl,

alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted

10   phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, nitro,

hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy,

substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted

phenylthio, arylthio, substituted arylthio, heteroarylthio, substituted

heteroarylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted

15   carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido,

substituted amido, sulfinyl, substituted sulfinyl, sulfonyl, substituted

sulfonyl, sulfonic acid, substituted sulfonic acid, phosphonato, substituted

phosphonato, phosphoramide, polyaryl, substituted polyaryl, C1-C20 cyclic,

substituted C1-C20 cyclic, heterocyclic, substituted heterocyclic, aminoacid,

20   peptide, or polypeptide group.

        The compound can form a chelate with a main group or transition

metal atom. Representative useful metal atoms are gallium (Ga), aluminum

(Al), cadmium (Cd), ruthenium (Ru), rhodium (Rh), platinum (Pt), palladium

(Pd), osmium (Os), iridium (Ir), iron (Fe), cobalt (Co), zinc (Zn),

25   molybdenum (Mo), titanium (Ti), manganese (Mn), chromium (Cr), nickel

(Ni), magnesium (Mg), copper (Cu), indium (In), vanadium (V) or vanadium oxide (VO), silver (Ag), gold (Au), or tin (Sn). The metal atom can have various leaving ligands. The leaving ligands can be any charged or uncharged ligands. In one embodiment, the ligand is Cl⁻. In another

5   embodiment, the ligand is an ether. In one embodiment, the metal atom is Cu, Fe, Mn, Ni, Zn, or VO. Typical pharmaceutically acceptable salts include H+, Na+, Li+, K+, Ca++, Mg++ and protonated amines and heterocyclic amines.

The compounds are formulated for adminitration to a mucosal

10   surface, such as to the female genital tract or rectum. The composition can also be used in combination with any number of other active agents or drugs, including antibiotics, virucidal or antiviral compounds, antifungals, immunomodulatory compounds, and contraceptives.

15   **Brief Description Of The Drawings**

Figure 1 shows the structures of phthalocyanines and porphyrazines. PcS is sulfonated phthalocyanine; PcC is carboxylated phthalocyanine; Alcian Blue, Alec Blue and Reactive Blue 15 are representative cationic phthalocyanine. The core structure of a porphyrazine is shown. M can be

20   H2 or a metal in all structures; porphyrazines can also have a central metal.

Figure 2 shows the synthesis of sulfonated phthalocyanines via protected sulfonic acids.

Figure 3 demonstrates the synthesis of an ABBB phthalocyanine via a subphthalocyanine.

25   Figure 4 shows exemplary phthalocyanines bearing OH groups.

Figure 5 shows the activity of sulfonated phthalocyanines against HIV-1 IIIB (percent virus inactivated).

Figure 6 shows the kinetics of inactivation of HIV-1 IIIB, percent of virus inactivated over time in minutes. Compounds at a final concentration

30   of 50 μg/ml were mixed with virus and incubated at various time intervals (5, 15, 30, 45, 60 min), diluted 1:10 with complete medium, and infectivity titers determined.

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Figure 7 shows the activity (percent virus inactivated) of selected phthalocyanines as a function of concentration. Virus samples were mixed with phthalocyanines at the indicated concentrations, and infectivity titers determined.

5   Figure 8 shows the percent inhibition of gp120-CD4 binding by various compounds. A 96-well plate coated with soluble CD4 was incubated with HIV-1 IIIB gp120 in the presence or absence of compounds for 1 hr at room temperature. After extensive washes the bound gp120 was detected by anti-gp120 peroxidase-conjugated antibodies. Results are given as % of

10   gp120 binding compared to untreated gp120 sample (100%).

Detailed Description Of Invention

Pharmaceutical phthalocyanines and/or porphyrazines compositions for preventing or treating viral and sexually transmitted diseases ("STDs") and the method of using the phthalocyanine and/or porphyrazine

15   compositions are provided herein. The pharmaceutical composition contains a synthetic phthalocyanine and/or porphyrazine or a metal complex thereof in an amount effective to inactivate a virus, preferably prior to infection. The composition may optionally include one or more pharmaceutically effective agents such as antibiotics, virucidals, antivirals, antifungals,

20   immunostimulants, and substances which are effective in inactivating viruses.

Definitions

The term sexually transmitted diseases ("STDs") as used herein refers to any viral diseases which can be transmitted via sexual behaviors.

25   Exemplary STDs include HIV infection and AIDS, infections caused by hepatitis B and/or C viruses, infection caused by papilloma viruses, genital herpes, genital warts, and syphilis.

Phthalocyanines are a class of compounds having a planar conjugated π-stack system (Figure 1). Phthalocyanines have eight nitrogen atoms and

30   eight carbon atoms forming a 16-member cyclic structure. The eight nitrogen atom provide an excellent environment for the molecule coordination to metal atoms. Porphyrazines are homologues of

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phthalocyanines in which the benzene rings are missing (Figure 1)
(Kobayashi "*meso*-Azaporphyrins and their analogs" in: *The Porphyrin
Handbook*, edited by K. M. Kadish and K. M. Smith, San Diego:Academic
Press, p. 301-360 (2000)). This planar conjugated system has 26 B electrons.

5   Like the phthalocyanines, porphyrazines are very stable. They can be
sublimed above 350 °C. Many metalloporphyrazines are also very stable.
For example, complexes with 3d metals (Cu, Co, Ni, Zn) dissociate only
slowly in hot concentrated sulfuric acid (Khelevina, et al., *J. Porph. Phthalo.*
4 (5):555-563 (2000)). The advantages of porphyrazines are that the smaller

10   central core gives a less hydrophobic species, with slightly different
placements of the side chains (see, for example, Kobayashi 2000; Khelevina,
et al., 2000).

I.   **Formulations**

    A.   **Phthalocyanines and Porphyrazines**

15   Phthalocyanines (Pcs) are being studied intensively in conjunction
with their use as photodynamic agents in chemotherapy and in the
phototherapeutic destruction of pathogens. Photodynamic tumor therapy
involves injection of a photosensitizing agent with a propensity to localize in
tumors, waiting for some hours (up to a few days) for maximum tumor

20   localization, and irradiation of the photosensitizing agent at the tumor site.
There are three major differences between photodynamic therapy and the
microbicide described herein. First, photodynamic therapy, by nature,
demands that the phthalocyanine be light sensitive. Light is not required for
the method described herein. Second, photodynamic agents are used

25   systemically and microbicides are used topically. Third, the local use of
microbicides prevent an initial infection of a STD pathogen. However, the
preclinical and early clinical studies on photodynamically active
phthalocyanines are helpful in assessments of safety and the possible side
effects of Pcs. The most widely studied Pcs for photodynamic therapy are

30   the aluminum sulfonated Pcs (AlPcS) (see, e.g., Luk'yanets, *J. Porphyrins
and Phthalocyanines* 3:424-432 (1999)); (Vakoulovskaia et al., Proc. SPIE
4059:32-38 (1999)); (Vakoulovskaia et al., Proc. SPIE 2924:39-313 (1996);
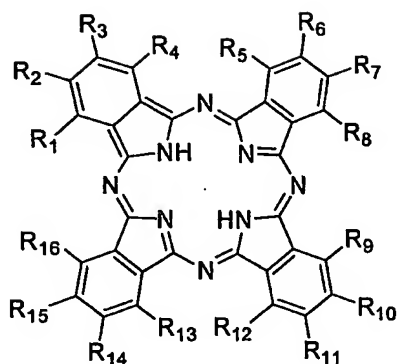
Stranadko et al., Proc. SPIE 3191:237-242 (1997)), (Zavodnov, Proc. SPIE
2625:482-483 (1997)), (Kharnas, Proc. SPIE 2625:449-450 (1997)),
(Vakoulovskaia et al., 1999). Other phthalocyanines which have been cited
as being in clinical trials for phototherapy of cancer include zinc(II)

5   phthalocyanine (Hadjur et al., J. Photochemistry and Photobiology B-
Biology 38:196-202 (1997)). The silicon phthalocyanine
HOSiPcOSi(CH₃)₂(CH₂)₃N(CH₃)₂ (Pc4, NSC 67418) is also entering clinical
trials (Egorin et al., Cancer Chemotherapy and Pharmacology 44:283-294
(1999); Allen, Sharman, and van Lier, Chemical Review 99:2379-2450

10   (2001)). Pc4 has passed the Decision Network III at the National Cancer
Institute (for cancers with estimated new cases per year below 200,000).

There have been a number of studies of photoinactivation of viruses
with phthalocyanines. These have been directed toward making the blood
supply safer for transfusions. Treatment of red blood cells with

15   phthalocyanines and red light leads to inactivation of enveloped viruses such
as vesicular stomatitis virus (VSV) (Moor et al., 1999), sindbis virus
(Rywkin et al., Photochemicstry and Photobiology 60:165-170 (1994)), HIV
(Horowitz et al., Transfusion 31:102-108 (1991); Margolis-Nunno et al.,
Transfusion 36:743-750 (1996); Ben-Hur, Oetjen, and Horowitz,

20   Photochemistry and Photobiology 54:703-707 (1997); Zmudzka et al.,
Photochemistry and Photobiology 65:461-464 (1997)) and herpes simplex
virus (HSV) (Rywkin et al., 1994; Smetana et al., J. Photochemistry and
Photobiology B-Biology 44:77-83 (1994)). All involve photoactivation of
the phthalocyanine to produce species (singlet oxygen or free radical) which

25   kill the virus. Virucidal activity depends substantially on both the
substituents on the periphery of the Pc as well as on the central metal ion.
Allen et al., Photochemistry and Photobiology 5:161-169 (1995) have
measured the Pc concentration (μM) required to photoinactivate 5 logs of
free vaccinia virus in an RBC suspension. They observed that virucidal

30   activity increased in the order Ga(III) < Al(III) < Zn(II); this may have
largely to do with the photophysical properties of the chelates. The addition
of a hydrophobic *tert*-butyl group to the unsubstituted ring of the Pc also
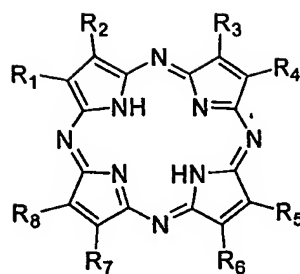
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enhanced the virucidal activity 10-fold. Finally, placement of two sulfonates on one side of the Pc gave 4-fold higher activity than the isomer in which the sulfonates were on opposite sides of the Pc. These observations indicate that Pcs with greater amphiphilicity bind more strongly to the virion. The anionic

5   aluminum phthalocyanine tetrasulfonate (AlPcS4) as well as the cationic silicon phthalocyanines $HOSiPcOSi(CH_3)_2(CH_2)_3N(CH_3)_2$ (Pc4), and $HOSiPcOSi(CH_3)_2(CH_2)_3N^+(CH_3)_3$ I^- (Pc5) were able to inactivate 10^5 infectious doses of cell-free HIV upon illumination with red light. However, of the three Pcs, only the cationic Pc4 was effective in inactivating actively

10   replicating HIV in infected cells.

There is evidence that inhibitors bind to components of the virion and prevent infection. For example, the compounds can bind to certain sites on the surface glycoprotein subunit (SU) gp120 and block its functions (Neurath et al., Antiviral Chem. Chemother. 3:55-63 (1992); Neurath et al., J.

15   Molecular Recognition 8:345-357 (1995)). These studies indicate the presence of non-neutralizing and neutralizing domains of gp120 as well as a silent face of the glycoprotein. Another open surface appears to be the receptor-binding region of HIV-1 gp120. These sites represent potential targets for the binding of virucidal compounds. The virucidal compounds

20   may also conformationally alter the protein or possibly block membrane fusion mediated by the gp120/gp41 complexes of the envelope protein.

The composition disclosed herein contain one or more compounds and/or their pharmaceutically acceptable salt, the compound having the following structure:

25

Formula I   Formula II

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16}

taken independently or together can be hydrogen, alkyl, substituted alkyl,

alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted

5   phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, nitro,

hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy,

substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted

phenylthio, arylthio, substituted arylthio, heteroarylthio, substituted

heteroarylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted

10   carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido,

substituted amido, sulfinyl, substituted sulfinyl, sulfonyl, substituted

sulfonyl, sulfonic acid, substituted sulfonic acid, phosphonato, substituted

phosphonato, phosphoramide, polyaryl, substituted polyaryl, C1-C20 cyclic,

substituted C1-C20 cyclic, heterocyclic, substituted heterocyclic, aminoacid,

15   peptide, or polypeptide group.

Compounds of Formulae I-II can chelate with any of the main group

or transition metals to form a metallocomplex. Metal atoms useful for

forming the metallocomplex with any of the compounds of Formulae I-II are

generally charged, though sometimes uncharged metal atoms may be

20   useable. Representative metal ions are any ions derived from gallium (Ga),

aluminum (Al), cadmium (Cd), ruthenium (Ru), rhodium (Rh), platinum (Pt),

palladium (Pd), osmium (Os), iridium (Ir), iron (Fe), cobalt (Co), zinc (Zn),

molybdenum (Mo), titanium (Ti), manganese (Mn), chromium (Cr), nickel

(Ni), magnesium (Mg), copper (Cu), indium (In), vanadium (V) or vanadium

oxide (VO), silver (Ag), gold (Au) or tin (Sn). In one embodiment, the metal atom is Cu, Fe, Mn, Ni, Zn, or V.

The metal atoms may have neutral or ionic ligands. Exemplary neutral ligands include H_2O, pyridine, imidazoles, NH_3, alkylamines, ethers,

5   oxygen, amino acid or peptide esters, phosphines, and alcohol. Other neutral ligands commonly used in coordination chemistry may also used. Exemplary ionic ligands can be negative charged ligands such as Cl^-, NO_2^-, CN^-, RS^-, and terminal N-bound amino acids or peptides. In general, the complexes formed of any of the disclosed compounds are more exchange

10   labile than their counterparts with the same metal but with other ligands attached. Also, alkyl or aryl ligands can be used.

Representative pharmaceutically acceptable salt is a salt of the compound or metallocomplex of the compound with a cation such as H+, Na+, Li+, K+, Ca++, Mg++, protonated amines and heterocyclic amines, and

15   quaternary ammonium including, but not limited to, alkyl and aryl or alkyl and aryls containing heteroatoms, such as oxygen, nitrogen and halides.

The compounds disclosed herein are capable of blocking early stages of the virus life cycle. For example, the disclosed compounds can block the initial interaction of the viral envelope glycoproteins with the CD4 receptor

20   as well as specific chemokine coreceptors for virus entry, the subsequent membrane fusion event which involves the N-terminal hydrophobic domain and heptad repeats of the transmembrane (TM) protein subunit, and the initiation of viral biosynthetic processes which result from the virion reverse transcriptase and integrase.

25   The phthalocyanine and porphyrazine compounds defined in Formulae I and II and their respective metallocomplexes do not encompass the phthalocyanine and porphyrazine compounds described in U.S. Patent Nos. 5,109,016 and 5,192,788. In particular, U.S. Patent Nos. 5,109,016 and 5,192,788 describe the following phthalocyanine and porphyrazine

30   compounds which were tested as effective for inhibition of HIV viruses and/or HSV viruses: copper phthalocyanine tetrasulfonic acid tetrasodium salt; nickel phthalocyanine tetrasulfonic acid; copper phthalocyanine 3,4',4'',4'''-tetrasulfonic acid; copper phthalocyanine; copper-4,4',4'',4'''-

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tetraaza29H, 31H-phthalocyanine; cobalt phthalocyanine; reactive blue 15; and silicon phthalocyanine dichloride. The most preferred compound is copper phthalocyanine tetrasulfonic acid available as a mixture from Aldrich.

Synthesis of phthalocyanine and porhyrazine compounds

5   (i)   Phthalocyanines and porphyrazines

Phthalocyanines (Pcs) and porphyrazines (Figures 1), the compounds of formulae I and II respectively, can be readily formed following procedures available in the art. In general, the formation of a Pc involves condensation of a phthalic acid derivative, which can be phthalic acid itself, the anhydride,

10   the nitrile, the cyano benzamide, the imide or the 1,3-diiminoisoindoline. Leznoff described 19 synthetic variations approximately a decade ago (Leznoff, "Synthesis of Metal-Free Substituted Phthalocyanines" In Phthalocyanines: Properties and Applications" (C.C. Leznoff and A.B.P. Lever Eds.) pp. 1-54 (VCH:New York) (1989); recent reviews on synthesis

15   include those by Torres, J. Porphyrins and Phthalocyanines 4:325-330 (2000), McKeown, Phthalocyanine materials: Synthesis, structure, and function (Cambridge University Press: Cambridge, U.K.) (1998) and the detailed review by Hanack (Hanack, M., H. Heckmann, et al., "Phthalocyanines and related compounds" in Methods of Organic Chemistry

20   (Houben-Weyl), E. Schumann, New York, Georg Thieme Verlag Stuttgart. E 9d: 717-846 (1998)). Many Pcs are synthesized from starting materials bearing a single substituent at the 4(5) or 3(6) position. Because the condensation can occur with each unit in the "clockwise" or "anticlockwise" direction, there are four constitutional isomers. A statistical mixture

25   comprises 12.5% each of the D_{2h} and C_{4h} isomers as well as 25% of the C_{2v} isomer and 50% of the C_s isomer. However, many reactions do not give a statistical mixture, due to charge distribution considerations in the intermediate steps of the synthesis as well as to steric hindrance in some cases. In some instances, it has been possible to separate and characterize

30   the isomers. All syntheses starting with 4(5)-substituted phthalonitriles should be regarded as giving a statistical mixture of the 4- and 5- isomers unless there is unambiguous experimental evidence to the contrary (Leznoff, 1989).

The following description describes various exemplary methods of making phthalocyanines.

Syntheses via crossed condensation

Hydrophobicity in general, and the placement of the charges in particular, have a significant effect on biological activity of phthalocyanines, as seen in studies of photodynamic anticancer and virucidal activities. The most promising candidates can be identified from biological studies. Additional related derivatives can be synthesized via cross condensation reactions. Crossed condensations of two phthalocyanine precursors A and B give a mixture comprising the AAAA, AAAB, ABAB, AABB, ABBB and BBBB products.

The substituents on the uncharged rings designed to promote ease of isolation of the structures. *tert*-Butyl groups in phthalocyanines help reduce aggregation of the structures and thereby facilitate isolation of the products. Naphthyl substituents give products with slightly different optical signatures, which facilitates characterization. Structures with identical substituents at the 4- and 5-position (e.g., resulting in a Pc with two identical side chains on one ring) would give fewer isomers. These can be synthesized to the extent that starting materials are readily available. On a large scale, separations can be achieved best if the syntheses are designed to give components with as different physical characteristics as possible. The role of protecting groups in this, especially for phenolic derivatives, is discussed below.

Synthesis of negatively-charged phthalocyanines – sulfonates

Synthesis of sulfonated phthalocyanines can be achieved by three major routes: synthesis from sulfophthalic acids, synthesis from protected sulfophthalic acids, and synthesis from precursors bearing a sulfonate group off the ring periphery. All of this chemistry is well developed in the literature and can be readily extendable to the paramagnetic central ions (see, for example, Bekâroglu, "Synthesis of phthalocyanines and related compounds" in *Journal Of Porphyrins And Phthalocyanines* 4, 465-473 (2000); de la Torre, G., Claessens, C. G., and Torres, T., "Phthalocyanines: The need for selective synthetic approaches" in *European Journal Of*

Organic Chemistry 2821-2830 (2000); Torres, "Perspectives in the selective

synthesis of phthalocyanines and related compounds" in *Journal Of*

Porphyrins And Phthalocyanines **4**, 325-330 (2000); Luk'yanets,

"Phthalocyanines as photosensitizers in the photodynamic therapy of cancer"

5   in *Journal Of Porphyrins And Phthalocyanines* **3**, 424-432 (1999); Hanack,

M., Heckmann, H., and Polley, in "Methods in Organic Chemistry (Houben-

Weyl), E9ds" pp. 717-842 (1998); J. H. Weber and D. H. Busch,

"Complexes derived from strong field ligands. XIX. Magnetic properties of

transition metal derivatives of 4,4',4'',4'''- tetrasulfophthalocyanine" in

10 *Inorg.Chem.* 4:469-471 (1965)).

Synthesis from sulfophthalic acids

Well-characterized sulfonated Pcs are often made via condensation of

sulfophthalic acid derivatives (Weber and Busch, 1965). The sodium salt of

4-sulfophthalic acid can be synthesized from 2-naphthalenesulfonamide

15 (Weber and Busch, 1965). The 1-naphthalenesulfonamide gives the 3-

sulfophthalate, which can also be used in condensations (Weber and Busch,

1965). Sulfophthalic acids can also be used in crossed condensations

(Margaron et al., J. Photochemistry and Photobiology B-Biology 14:187-199

(1992)).

20 ### Synthesis from phthalonitriles bearing a sulfonic acid removed from the ring

The condensation is also successful when the sulfonic acid is

removed from the phthalocyanine ring. For example, Wöhrle and co-

workers treated nitrophthalonitrile with *p*-hydroxybenzenesulfonic acid to

25 give the substituted phthalonitrile which was then condensed to the

phthalocyanine (Kliesch et al., Liebigs Annalen 1269-1273 (1995)). Yields

appear to be about the same as other phthalocyanine syntheses, opening up a

wide variety of possible structures, limited largely by the sulfonic acid

alcohols available. Examples of commercially available starting materials

30 include $HO(CH_2)_nSO_3Na$ where n = 2 and 3. The homologs with n = 4 and 5

are readily synthesized (White and Lim, J. Org. Chem. 52:2162-2166

(1987)). Crossed condensation can also be used to make a variety of Pcs

with the sulfonic acid removed from the phthalocyanine core, e.g., in work of

Wöhrle and co-workers (Kliesch et al., 1995). In related work with thioether derivatives, Tabata et al. J. Porphyrins and Phthalocyanines 4:278-284 (2000) were able to make Pcs with as many as 15-16 sulfonate groups per molecule.

Synthesis from protected sulfophthalic acids

5　　　　Protected sulfonic acids offer substantial advantages in the synthesis of phthalocyanines. They allow purification to be performed with silica columns on an organic-soluble material. If deprotection goes in high enough yield, the sulfonic acid derivatives can be generated cleanly. Recently, Leznoff and collaborators investigated six different sulfonamide protecting

10　　groups for robustness during the phthalocyanine synthesis as well as high yield in the deprotection step (Li et al., Canadian J. of Chemistry-Revue Canadienne De Chimie 77:138-145 (1999)). The pyrroylsulfonyl and indolylsulfonyl groups proved to be the best. Figure 2 shows the overall synthesis and yields. Recently, van Lier and co-workers have used this indole

15　　protection group in cross-condensation reactions, focusing on trisulfonated derivatives (Brasseur et al., Photochemistry and Photobiology 69:345-352 (1999) (1999a); Brasseur, et al., Br. J. Cancer 80:1533-1541 (1999) (1999b). Deprotection of the three sulfonates with LiOMe in MeOH/THF occurred in an overall yield of 80 - 85% for 9 different derivatives (Tian et al.,

20　　Tetrahedron Letters 41:8435-8438 (2000)).

Sulfonated derivatives via subphthalocyanines

Another preferred technique for the synthesis of ABBB-type phthalocyanines involves the ring expansion of a subphthalocyanine. Subphthalocyanines are homologs of phthalocyanine with three isoindole

25　　units and boron as the central atom (Torres, 2000). This is allowed to condense with a fourth ring to give the ABBB product. The products of this method depend on the reaction conditions and structure of the starting materials; scrambling is possible under certain conditions (de la Torre et al., European Journal of Organic Chemistry 2821-2830 (2000)). However, other

30　　sets of conditions can give the desired ABBB compound cleanly. Shown in Figure 3 is an example of a water-soluble, unsymmetrical, trisulfonated zinc phthalocyanine (ZnPcS3) as the single product of the ring expansion of boron tri(4-sulfo)subphthalocyanine (SubPc) (Kudrevich et al., J. Med.

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Chem. 40:3897-3904 (1997)); Kobayashi, J. Porphyrins and Phthalocyanines
3:453-467 (1999); Kobayashi et al., J. Am. Chem. Soc. 121:9096-9110
(1999)).

Sulfonation of a pre-formed phthalocyanine

5   Sulfonation of a preformed Pc is well established and can be readily
performed by one of ordinary skill in the art. Mixtures of the mono-, di-, tri-
and tetrasulfonated products as well as mixtures of positional isomers in each
of these products are obtained (Linstead and Weiss, J. Chem. Soc. 2975-2981
(1950); Ali et al., Photochemistry and Photobiology 47:713-717 (1988)).

10   Various degrees of sulfonation and various isomers may have different
pharmaceutical properties, as found by others in studies of photodynamic
therapy (Kessel et al., Photochemistry and Photobiology 45:787-790 (1987);
Berg et al., Photochemistry and Photobiology 52:481-487 (1990) (1990a);
Berg et al., Photochemistry and Photobiology 52:775-781 (1990) (1990b))

15   and in the examples described below. However, mixtures of sulfonated
phthalocyanines containing molecules with different numbers of sulfonate
groups as well as different positions of the sulfonate on the periphery of the
Pc skeleton are useful.

Synthesis via conjugates of sulfonates

20   The conjugation of phthalocyanines with other molecules opens up
vast possibilities for new structures. Conjugates of tetrapyrroles, including
phthalocyanines, with a wide variety of molecules have been studied in
conjunction with the use of photosensitizers for anticancer therapy.
Conjugates include those with lipid-like molecules, antibodies, and

25   macromolecular internalizable ligands such as conA, insulin, transferrin,
epidermal growth factor and peptide signals for targeting to various
intracellular compartments. A very recent review in this area has
approximately 300 references (Sobolev et al., Progress in Biophysics &
Molecular Biology 73:51-90 (2000)). van Lier and colleagues have recently

30   published detailed procedures for making conjugates of sulfonated Pcs in
conjunction with their work on bovine serum albumin conjugates (Brasseur
et al., 1999a, 1999b). For example, they treated AlPcS$_4$ with chlorosulfonic
acid followed by thionyl chloride to give 90% of the chlorosulfonic acid

derivative. This activated AlPc-tetrasulfonyl chloride is stable for at least two months at -20 °C (Brasseur et al., 1999a, 1999b). The AlPc-tetrasulfonyl chloride (1.0 g scale) was then treated with 6-aminohexanoic acid and sodium carbonate. The reaction mixtures were purified in 200 - 400 mg

5   batches by reverse-phase HPLC. Very good separation was achieved for the mono- and bis-derivatives. A wide variety of amines can then be used for conjugation; the derivatives chosen would depend on the desired properties of the final product, with our first efforts concentrated on making derivatives with different hydrophobicities.

10   _Synthesis of phosphate, phosphonate and carboxylate_

derivatives

Derivatives with phosphoric acids offer yet another series of structures. These have been far less well studied than the sulfonate derivatives. The compound bearing a phosphonate moiety directly on the

15   phthalocyanine ring has been synthesized by van Lier and co-workers (Sharman et al., Tetrahedron Letters 37:5831-5834 (1996)). This group has also made a phthalocyanine with a phosphate group removed from the Pc periphery (Boyle and van Lier, Synthesis-Stuttart 1079-1080 (1995)). A series of Al, Zn, Si and Sn Pcs having 4 to 8 diethoxy or

20   dihydroxyphosphinylmethyl groups has also been synthesized (Luk'yanets, 1999). Many metal derivatives of the 4,4',4",4" tetracarboxylic acid Pc are known (Shirai et al., Makromol. Chem. 181:575-5834 (1980)). It is also possible to isolate compounds in which only one of the carboxylic acids is derivatized (Kobayashi et al., J. Chem. Soc. Dalton Transactions 10:2107-

25   2110 (1984)). Phthalocyanines with carboxylic acids not directly attached to the ring can be made using appropriate phthalonitrile precursors (Cook et al., J. Chem. Soc. Chem. Comm. 1148-1150 (1987)).

Hydroxyphthalocyanines

Selected phenolic Pcs can be used as attachment points for various

30   conjugates (see, for example, Sobolev et al., 2000; Li and Ng, Tetrahedron Letters 42:305-309 (2001)). For example, Figure 4 shows some selected examples of phthalocyanines bearing OH groups. The example with OH groups at the 4(5) positions can be a mixture of four isomers (far left). The

example with OH groups at the 3(6) positions is usually formed as a single

isomer (from protected OH groups). The OH can also be on one of the side

chains. Partially substituted derivatives are also available (e.g., the right-

hand structure). The 4(5)-tetrahydroxyPc (left-hand structure of Figure 4)

5   can be synthesized by, for example, deprotection of the Pc with p-n-

BuPhCH$_2$ or Ph$_2$CH protecting groups (Leznoff et al., Canadian J. of

Chemistry-Revue Canadienne De Chimie 72:1990-1998 (1994)).

Condensation of the isomeric 3-p-n-BuPhCH$_2$O-substituted phthalonitrile in

the presence of a metal such as zinc gives the phthalocyanine in high yield;

10   deprotection then generates the tetrahydroxyphthalocyanine (see, for

example, Leznoff et al., 1994).

Other derivatives in this class would have the alcohol connected to

the phthalocyanine core via a spacer chain. For example, Pcs substituted

with primary alcohol side chains made following the procedure of Boyle et

15   al., who made zinc phthalocyanines substituted with four propanol or

hexanol groups (Boyle et al., Synlett 5:351-353 (1993)).

Procedures such as crossed condensation can be used to make Pc

derivatives with OH groups on only some of the peripheral positions. Hu et

al. J. Med. Chem. 41:1789-1802 (1998) have recently published procedures

20   to achieve this using various benzyl and substituted-benzyl-protected

phenolic precursors. If the protecting groups are bulky enough, it is possible

to isolate various substituted Pcs by gel permeation (size exclusion)

chromatography. For example, prevention of 4-(diphenylmethoxy)

phthalonitrile and phthalonitrile (1:10 ratio) with DBU (1,8-

25   diazabicyclo[5.4.0]undec-7-ene) in 1-butanol at 100°C for 1 h, followed by

addition of excess zinc acetate, gave the Zn(II) 2-

(diphenylmethoxy)phthalocyanine in 42% yield. The same two starting

materials in a 10:1 ratio gave the trisubstituted Pc in 40% yield. Cleavage of

the protecting groups using trifluoroacetic acid and 1,2,4,5-

30   tetramethylbenzene gave the corresponding hydroxyphthalocyanines in 90%

(mono) and 77% (tris) yields (Hu et al., 1998). Conjugates of

hydroxyphthalocyanines should be readily synthesized in fairly good yield

(Li and Ng, 2001). The chemistry of phenolic phthalocyanines has been

worked out in substantial detail on a relatively large scale. The compounds
are separated in their protected forms, which are amenable to classic
chromatography on silica or alumina. For organic compounds, this tends to
give better separation on a larger scale in comparison with media, which are

5   used to effect separations of compounds in aqueous solution. In addition,
large protecting groups can be chosen to maximize the difference in physical
characteristics of the Pcs in the mixture, assisting the separation.

Other approaches to unsymmetrical phthalocyanines

As structure-activity relationships are developed in the biological
10   testing, it may become clear that specific types of unsymmetrical
phthalocyanines are desired as therapeutic candidates. Routes such as the
AABB syntheses via half-Pc intermediates, three-quarter phthalocyanines
and hemiporphyrazines are just some of the possibilities for unsymmetrical
phthalocyanines. Recent reviews give leading references (Hanack,
15   Heckmann, and Polley, 1998; de la Torre, et al. 2000; Torres, 2000).
Unsymmetrical phthalocyanines can also be formed via a palladium-
catalyzed coupling reaction between and iodophthalocyanine and acetylenic
derivatives. The coupling reaction goes in high yield (Ali, 1997; Tian, et al.,
2000).

20   The substituents, as well as the overall structure, of the compounds of
formulae I and II disclosed herein can be neutral, positively charged or
negatively charged. Charged structures have counterions, and many
counterions and combinations of counterions are possible. The disclosed
compounds can be covalently attached to other molecules, for example, a
25   nucleobase or oligonucleotide (Koval et al. Nucleosides Nucleotides &
Nucleic Acids. 20:1259-1262 (2001), Li et al. Tetrahedron Lett. 42:305-309
(2001)), a peptide or protein (Brasseur et al. Photochem.Photobiol. 69:345-
352 (1999); Carcenac et al. Photochem.Photobio. 70:930-936 (1999);
Lutsenko et al. Tumor Biology 20:218-224 (1999)), a sugar or sugar
30   derivative (Maillard et al. J.Am.Chem.Soc. 111:9125-9127 (1989)), a lipid
(Morgan et al. Photochem.Photobiol. 60:486-496 (1994)) or a polymer
(Brasseur et al. Br.J.Cancer. 80:1533-1541 (1999)). They can have an

attached molecular superstructure. The conjugation of the ring can be altered by addition of one or more substituent.

(ii)   Metal complex of compounds of Formulae I-II

Compounds of Formulae I-II can react with any of the main group or

5   transition metals. Metal atoms useful for forming a complex with any of the compounds of Formulae I-II are generally charged, though sometimes uncharged metal atoms may be useable. Representative metal ions are any ions derived from Ga, Fe, Cu, Co, Ni, Pt, Os, Ru, Rh, Ir, Pd, Mn, V, Cr, Zn, Ag, Au, Cd, Ba, Al, Ti, or Sn. In one preferred embodiment, the metal ions

10   are derived from Cu, e.g., Cu(II). In another preferred embodiment, the metal ions are derived from Fe, e.g., Fe(III).

The metal atoms may have neutral or ionic ligands. Exemplary neutral ligands include H_2O, pyridine, imidazoles, NH_3, alkylamines, ethers, oxygen, amino acid or peptide esters, phosphines, and alcohol. Other neutral

15   ligands commonly used in coordination chemistry may also used. Exemplary ionic ligands can be negative charged ligands such as Cl^-, NO_2^-, CN^-, RS^-, terminal N-bound amino acids or peptides).

The substituents, as well as the overall structure, of the compounds disclosed herein can be neutral, positively charged or negatively charged.

20   Charged structures have counterions, and many counterions and combinations of counterions are possible. The disclosed compounds can be covalently attached to other molecules, for example, a nucleobase or oligonucleotide (Koval et al. Nucleosides Nucleotides & Nucleic Acids. 20:1259-1262 (2001), Li et al. Tetrahedron Lett. 42:305-309 (2001)); a

25   peptide or protein (Brasseur et al. Photochem. Photobiol. 69:345-352 (1999); Carcenac et al. Photochem. Photobiol. 70:930-936 (1999); Lutsenko et al. Tumor Biology 20:218-224 (1999)), a sugar or sugar derivative (Maillard et al. J. Am. Chem. Soc. 111:9125-9127 (1989)), a lipid (Morgan et al. Photochem. Photobiol. 60:486-496 (1994)) or a polymer (Brasseur et al. Br.

30   J. Cancer. 80:1533-1541 (1999)). They can have an attached molecular superstructure. The conjugation of the ring can be altered by addition of one or more substituent.

Selection of the comp unds for inactivation f viral pathogens

Test of virucidal activities of phthalocyanine and

p rphyrazine compounds

One can screen the compositions for inactivation of viral pathogens

5   such as HIVs or HSVs by various experimental techniques. In one

embodiment, the technique involves the inactivation of viral replication in

human peripheral blood mononuclear cells. The amount of virus produced is

determined by measuring the quantity of virus-coded reverse transcriptase

(an enzyme found in retroviruses) which is present in the culture medium.

10   Another technique involves measuring inactivation of purified reverse

transcriptase in a cell free system.

Hydrophobicity of the compounds

The relation between hydrophobicity of porphyrin, a class of

compounds closely related to phthalocyanines, and its biological activity has

15   been studied. There is documentation that the hydrophobic interactions of

the planar extended aromatic porphyrin ring help stabilize its interactions

with biomolecules (see, for example, Stephen J. Lippard and Jeremy M.

Berg, Principles of Bioinorganic Chemistry, University Science Books,

1994). Other significant interactions include hydrogen bonding and

20   electrostatic interactions of the peripheral substituents, and axial interactions

involving the metal (Lippard & Berg, 1994). The hydrophobicity of the

compounds disclosed herein can be evaluated by using, for example,

capillary electrophoresis and fluorescence assays. Therefore, by analyzing

the hydrophobicities of various compounds disclosed herein, it is possible to

25   establish the relationship between hydrophobicity and a particular structure

of the compound disclosed herein, thereby allowing the prediction of highest

possible hydrophobic interactions of the compound with a biomolecule.

Quantitative Structure Activity Relationship (QSAR) Analysis of

Biological Activity

30   QSAR can be used to provide guidance for the selection of the most

effective compounds disclosed herein for the prevention of STDs caused by

viral pathogens or AIDs caused by HIVs. QSAR has wide application in

guiding the design of new pharmaceutical agents (see for example, Karelson

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et al., Chem. Rev., 96:1027-1043 (1996); 3D QSAR in Drug Design: Ligand-Protein Interactions and Molecular Similarity, Hugo Kubinyi; Yvonne C. Martin; and Gerd Folkers (Eds.), Kluwer Academic Publishers, Dordrecht, Netherlands, Vol. 2, (1998); 3D Qsar in Drug Design: Recent Advances,

5   Hugo Kubinyi; Yvonne C. Martin; and Gerd Folkers (Eds.), Kluwer Academic Publishers, Dordrecht, Netherlands, Vol. 3, (1998)). Successful use of QSAR can substantially shorten the time needed to develop a new drug. For example, the most detailed, relevant example of QSAR guidance in the design of new porphyrins and metalloporphyrins as virucidal agents is

10   a study of porphyrin and metalloporphyrin anti-HIV-1 agents binding to the gp 120 V3 loop sequence (Debnath et al., J. Med. Chem. 37:1099-1108 (1994)). Approximately 20 porphyrins were tested as anti-HIV agents including various NPs and porphyrins in the TPP carboxylic acid family. Debnath et al., used comparative molecular field analysis (CoMFA)

15   (Kroemer et al., 1998; Norinder, 1998). Another approach is to derive molecular parameters from a number of sources and use these in a multiple linear regression to predict relative activity. Parameters might include the surface area, volume and polarizability of the compound (the ChemPlus module in HyperChem, Hypercube, Inc.) as well as the dipole moment,

20   LUMO and HOMO (and derived parameters) and net charge from the electrostatic potential (Gaussian).

        B.   Other Agents

        The formulation may optionally include, or be administered with, one or more other pharmaceutically active agents. Examples include antibiotics,

25   virucidals or virustatics, antivirals, antifungals, immunostimulants, and substances which may have other properties, such as contraceptives. These may be synthetic or natural drugs, natural or synthetic proteins, saccharides or polysaccharides, nucleic acid molecules, or combinations thereof. Examples include proteins such as antibodies, microbicidal polymers such as

30   a cyclodextrin, polyethylene hexamethylene biguanide, a polymer such as Buffergel, a seaweed polymer such as Carraguard, or antimicrobial peptides such as a definsin. In another embodiment, the pharmaceutically effective agent can be a drug that inactivates one or more viruses.

C.   **Pharmaceutically Acceptable Formulations**

The formulations will typically be administered to a mucosal surface to prevent infection with a STD or other viral pathogen and/or AIDs caused by HIV. The compounds have been established as not being useful in

5   preventing infection by HSV-1 and HSV-2.

The compounds are formulated to provide an effective amount of the compound described herein, a metallocomplex of the compound, a pharmaceutically acceptable salt of the compound, or a mixture thereof. The pharmaceutically effective amount varies with the type of STD. In one

10   embodiment the composition is used for HIV prevention, for which the effective amount of the compound is a concentration *in vitro* of less than or equal to 10 μM in the presence of a pharmaceutically acceptable carrier or diluent.

Some of the compounds are water soluble and may be administered

15   in sterile water or physiological saline or phosphate buffered saline (PBS). Many porphyrins are not water soluble and are preferably administered in pharmaceutically acceptable non-aqueous carriers including oils and liposomes. Solubility of the compounds can be increased by techniques known to those skilled in the art including introducing hydroxyl groups and

20   changing the counter ions.

The compounds are included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to exert a therapeutically useful inhibitory effect on HIV *in vivo* without exhibiting adverse toxic effects on the user. By "HIV inhibitory amount" is meant an amount of active

25   ingredient sufficient to exert an HIV inhibitory effect as measured by, for example, an assay such as the ones described herein.

There may also be included as part of the composition pharmaceutically compatible binding agents, and/or adjuvant materials. The active materials can be mixed with other active materials including

30   antibiotics, antifungals, other virucidals and immunostimulants which do not impair the desired action and/or supplement the desired action. The formulations can be administered by any route, for example, orally,

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parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form. A preferred mode of administration of the compositions described herein is topical or mucosal administration. A specifically preferred mode of mucosal administration is administration via female

5   genital tract. Another preferred mode of mucosal administration is rectal administration.

It is to be noted that dosage values also vary with the specific severity of the disease condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted to the

10   individual need and the professional judgment of the person administering or supervising the administration of the compositions. It is to be further understood that the concentration ranges set forth herein are exemplary. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

15   The phthalocyanine and/or porphyrazine compounds, a metallocomplex of the compound, pharmaceutically acceptable salts of the compound or metallocomplex, or a mixture thereof can be formulated as ointments, creams, gels, lotions, troches, suppositories, vaginal rings, liposomes, nanoparticulates, microspheres, and controlled release

20   formulations. Various polymeric and/or non-polymeric materials can be used as adjuvants for enhancing mucoadhesiveness of the composition disclosed herein. The polymeric material suitable as adjuvants can be natural or synthetic polymers. Representative natural polymers include, for example, starch, chitosan, collagen, sugar, gelatin, pectin, alginate, karya

25   gum, methylcellulose, carboxymethylcellulose, methylethylcellulose, and hydroxypropylcellulose. Representative synthetic polymers include, for example, poly(acrylic acid), tragacanth, poly(methyl vinylether-co-maleic anhydride), poly(ethylene oxide), carbopol, poly(vinyl pyrrolidine), poly(ethylene glycol), poly(vinyl alcohol),

30   poly(hydroxyethylmethylacrylate), and polycarbophil. Other bioadhesive materials available in the art of drug formulation can also be used (see, for example, Bioadhesion – Possibilities and Future Trends, Gurny and Junginger, eds., 1990).

The formulation may contain the following ingredients: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, corn starch and the like; a lubricant such as magnesium stearate or Sterotes; a
5   glidant such as colloidal silicon dioxide; and a sweetening agent such as sucrose or saccharin or flavoring agent such as peppermint, methyl salicylate, or orange flavoring may be added. When the dosage unit form is a capsule, it may contain, in addition to material of the above type, a liquid carrier such as a fatty oil. Other dosage unit forms may contain other various
10   materials which modify the physical form of the dosage unit, for example, as coatings. Tablets or pills may be coated with sugar, shellac, or other enteric coating agents. Materials used in preparing these various compositions should be pharmaceutically pure and non-toxic in the amounts used.

       Solutions or suspensions may also include the following components:
15   a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methylparabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or
20   phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

       Controlled release formulation, including implants and microencapsulated delivery systems may also be used. Biodegradable,
25   biocompatable polymers can be used, such as polyanhydrides, polyglycolic acid, collagen, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also useful as pharmaceutically acceptable
30   carriers. Methods for encapsulation or incorporation of compounds into liposomes are described by Cozzani, et al., Chem. Biol. Interact. 53:131-143 (1985) and by Jori, et al., Br. J. Cancer 48:307-309 (1983). These may also

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be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

II.   Methods of Preventi n of STDs or other Infections

Generally, the formulations can be used to prevent a viral infection

5   by administering to a human being an effective amount of at least one of phthalocyanines, porphyrazines, metal complexes thereof, pharmaceutically acceptable salts thereof, or a mixture thereof that inactivates a virus prior to an infection caused by the viruses being effected. Optionally, a pharmaceutically effective amount of one or more of other agents can be

10   used in combination with the one of phthalocyanines, porphyrazines, metal complexes thereof, or a mixture thereof.

The compound of formulae I-II or a metal complex thereof has broad-spectrum anti-viral activities. These compounds or metal complexes can be formulated for administration to individuals in need of prevention of STDs.

15   The formulations are preferably for local or regional delivery, for example, to the mucosa of the reproductive tract, or intestinal tract, but may also be formulated for systemic delivery. The formulation is designed to administer an amount of porphyrin effective to prevent infection of the STD. The time of administration is determined based on standard clinical criteria,

20   determined using other antibiotic or virucidal formulations, clearance rates, and STD to be treated.

The compositions can be used to prevent STDs caused by viral pathogens. Exemplary viruses include HIV viruses, hepatitis B and C viruses, and papilloma viruses. The pharmaceutically effective amount

25   varies with the type of STD. Typically, an effective amount of the porphyrin compound is less than or equal to 10 µM in the presence of a pharmaceutically acceptable carrier or diluent. The compounds described herein are included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to exert a therapeutically useful inhibitory effect in vivo

30   without exhibiting adverse toxic effects on the user. It is to be noted that dosage values also vary with the specific severity of the disease condition to be alleviated. It is to be further understood that for any particular subject,

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specific dosage regimens should be adjusted to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

Other agents which can be used in combination with the compounds

5   or metal complexes described herein include antibiotics, virucidals, antivirals, antifungals, immunostimulants, and substances which are effective in inactivating viruses. In one embodiment, the pharmaceutically effective agents include synthetic or natural drugs, natural or synthetic polymers, and antibodies. In one embodiment, the agent can be a microbicidal polymer

10 such as one of cyclodextrins, polyethylene hexamethylene biguanide, a polymer such as Buffergel, a seaweed polymer such as Carraguard, and antimicrobial peptide such as one of definsins. In another embodiment, the pharmaceutically effective agent can be a drug that inactivates one or more viruses.

15 The following examples further illustrate the phthalocyanine and Pporphyrazine compositions disclosed herein and the method of using thereof for inactivation of HIVs and pathogens causing STDs.

Example 1. Prevention of HIV-1 Infection by Phthalocyanines

<u>Materials and Methods</u>

20 *Phthalocyanines*

The cationic phthalocyanines tested were Alec Blue (Aldrich, St. Louis, MO) (PcCat(1)) and Alcian Blue 8GX (PcCat(2)) (Kodak). Patrea – original first sentence is OK – I should not have changed it. The Al and Co carboxyphthalocyanines (AlPcC and CoPcC) were from Midcentury

25 Chemicals (Posen, Illinois). The Co and Fe sulfonated phthalocyanines (Midcentury Chemicals) were made via the Weber-Busch synthesis (Weber and Busch, 1965), and thus are expected to be tetrasulfonates. The other commercial phthalocyanines are apparently mixtures with different extents of sulfonation as well as different positions of the sulfonate on the periphery

30 of the Pc skeleton. There was no obvious correlation between the activity of a given phthalocyanine and the number of components in the mixture. CuPcS(3,4',4'',4''') (Aldrich) is designated as CuPcS(1) and CuPcS(4,4',4'',4''') (Fluka, St. Louis, MO) is designated as CuPcS(2). The

free acid [CuPcS(H+)] and lithium salt [CuPcS(Li+)] of CuPcS were from
Frontier Scientific (Logan, Utah). NiPcS was from Aldrich; the rest of the
phthalocyanines were from Midcentury Chemicals. The aluminum and zinc
phthalocyanines were available in two sulfonation levels. AlPcS(1) and

5   ZnPcS(1) are the more highly sulfonated forms (presumably largely the
tetrasulfonates). AlPcS(2) and ZnPcS(2) are less sulfonated (listed as the
trisulfonated forms).

Cell lines

10   The mouse NIH/3T3 and human HEp2 cell lines were obtained from
the American Type Culture Collection (Manassas, VA). The recombinant
cell lines human MAGI, monkey sMAGI, mouse 3T3.T4, 3T3.T4.CCR5,
3T3.T4.CXCR4, and human T-cell lines CEMx174 and HUT78 were
obtained through the AIDS Research and Reference Reagent Program,

15   Division of AIDS (NIH). The human 293T cell line was kindly provided by
Dr. S. L. Lydy (Emory University, Atlanta, GA). NIH/3T3, HEp2, 3T3.T4,
3T3.T4.CCR5, 3T3.T4.CXCR4, MAGI, sMAGI, and 293T cells were
maintained in Dulbecco's minimal essential medium (DMEM) supplemented
with 10% fetal calf serum. HUT78 and CEMx174 cells were maintained in

20   RPMI 1640 medium supplemented with 10% fetal calf serum.

Viruses and plasmids

For construction of recombinant vaccinia viruses, plasmids pRB21
and vRB12 were kindly provided by Drs. Bernard Moss (National Institutes
of Health, Bethesda, MD) and David Steinhauer (National Institute for

25   Medical Research, London, UK). The 3'SHIV-89.6 plasmid was obtained
from Dr. J. Sodroski (Harvard Medical School, Boston, MA). Recombinant
vaccinia viruses expressing full length (VV-239env) and truncated (VV-
239T) SIVmac239 Env proteins were previously described by (Ritter, et al.,
Virology 197:255-264 (1993)), and VVenv1 expressing the BH10 Env

30   protein was described by Owens and Compans (Owens and Compans, J.
Virol. 63, 978-982 (1989)). A recombinant vaccinia virus encoding a
truncated Env protein of HIV-1 89.6 was constructed as follows. The HIV-1
89.6 truncated env gene was obtained by polymerase chain reaction (PCR)

amplification from the HIV-1 89.6 plasmid with the following primers: the
5′-primer introducing an EcoRI site 5′-
GAGAAGAATTCAGTGGCAATGAGAG TGAAGG-3′; the 3′ primer
introducing an Nhe I site and a premature stop codon after the codon for

5   amino acid (aa)17 in the cytoplasmic domain 5′ CCTGTCGGCTAGC
CTCGATCATGGGAGG AGGGTCTGAAACGATAATG. The PCR
product was then digested by EcoR I and Nhe I and ligated into EcoR I and
Nhe I – predigested pRB21 as a donor plasmid for vaccinia recombination.
The recombinant vaccinia virus was obtained by a plaque selection system

10   using a recipient vaccinia virus vRB12 described by Blasco and Moss
(Blasco and Moss, Gene 158:157-162 (1995)). The plasmid pIIIenv3-1
encoding the Env protein of the HXB2 strain of HIV-1 was obtained from
the AIDS Research and Reference Reagent Program, Division of AIDS
(NIH). The Tat-responsive HIV-LTR in pIIIenv3-1 was used to promote

15   expression of HXB2 Rev and Env. The helper plasmid pCMVtat was kindly
provided by Dr. Steven Bartz (Fred Hutchinson Cancer Research Center,
Seattle, WA). Virus-infected H9/HTLV-III$_B$ NIH 1983 cells were obtained
from the AIDS Research and Reference Reagent Program, and the
supernatant was used to infect HUT78 cells. HIV-1 IIIB virus was produced

20   by continued passage of infected HUT78 cells and virus stock was prepared
as described previously (Vzorov and Compans, J. Virol. 74:8219-8225
(2000)). To prepare HIV-1 89.6 virus, 293T cells were transfected with
p89.6 (from the NIH AIDS Research and Reference Reagent Program). At
48 hr post transfection, DMEM was removed and the cells were washed once

25   in RPMI. Then $2x10^6$ CEMx174 cells were added to a plate in 5 ml of RPMI
containing 10% fetal calf serum and cocultured overnight. The following
day, CEMx174 cells were removed from virus-producing 293T cells and
placed in T-25 flasks for continued passage. SIVmac1A11 virus stock was
described previously (Vzorov and Compans, 2000).

30   *Monoclonal antibodies, antisera, proteins*
SIM.2 and SIM.4 antibodies recognizing human CD4 and
recombinant soluble human CD4 were provided by the NIH AIDS Research

and Reference Reagent Program (NIH). The recombinant IIIB gp120 protein (baculovirus-expressed) was obtained from Intracel (Cambridge, MA). Anti-mouse IgG peroxidase conjugate was obtained from Sigma (St. Louis, MO).

Screening of phthalocyanines for activity against HIV-1

5   Phthalocyanine stock solutions were prepared at concentrations of 5 mg/ml, diluted 100-fold in growth medium, and mixed with virus stock. Samples were left in the dark at room temperature for 1 hr. For MAGI or sMAGI assays, 25 μl of virus/compound mixture was mixed with 225 μl of growth medium containing DEAE-Dextran (15 μg/ml) and 50 μl added to

10   wells with confluent monolayers of MAGI or sMAGI cells (on a 96 well plate). At 2 hr post infection, an additional 200 μl of complete DMEM was added. After three days virucidal activity was measured by removal of the media, fixation with 1% formaldehyde and 0.2% glutaraldehyde and staining with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). About 50-

15   60 separate blue nuclei per well were observed for the positive control. Scoring of blue nuclei in a 96-well format was greatly enhanced by using a planar lens [Olympus (Japan) 4x] to visualize the entire well. For determining virus titers we used RT (Roche), MAGI (Kimpton and Emerman, J. Virol. 66:2232-2239 (1992)) or sMAGI (Chackerian et al.,

20   Virology 213:386-394 (1995)) assays. Comparison of the number of blue cells in wells infected with compound-treated virus to the number found in wells infected with untreated virus was used to determine residual viral infectivity (expressed in percent).

gp120-CD4 binding assay

25   To investigate the possible effect of phthalocyanine compounds on binding of HIV-1 IIIB gp120 to CD4, a gp120-CD4 binding assay was developed. The assay was developed as a modification of a capture gp 120 ELISA kit (Intracel Corporation). Briefly, a 96-well plate was coated with soluble CD4 and 0.5 μg of HIV-1 IIIB gp120 per well was incubated in the

30   presence or absence of test compounds for 1 hr at room temperature. After 4 washes with buffer to remove unbound proteins, the bound gp120 was

detected by anti-gp120 peroxidase-conjugated antibodies and quantitated by
the protocol provided by the manufacturer.

Cell fusion assays

For cell fusion assays, three different expression systems: (1) a
5 recombinant vaccinia expression system, which is able to express high levels
of Env; (2) a plasmid expression system which is able to express Env
proteins in the absence of other HIV proteins or vaccinia proteins; and (3)
cells persistently infected with HIV-1 IIIB or HIV-1 89.6, were compared.
In the first system, HEp2 cells were infected at a m.o.i. of 5. After 24 hr
10 cells were collected, counted and about 2.5×10^3 cells were added to
3T3CD4CXCR4 or 3T3CD4CCR5 cell monolayers in 96-well plates in 100
µl of media in the presence or absence of the test compounds.

For the second assay 293T cells were transfected by the calcium
phosphate precipitation method with the plasmid pIIIenv3-1 expressing the
15 HIV-1 Env protein (HXB2 Env) with an LTR promoter, and cotransfected
with a helper plasmid pCMVTAT at a ratio of 10:1; or with plasmids
expressing SIV Env proteins using a CMV promoter as described above.
After 48 hr cells were collected and cocultured with uninfected cells as in the
previous assay.

20 As a third system HUT78 cells were persistently infected with HIV-1
IIIB or CEMx174 cells persistently infected with HIV-1 89.6. The infected
cells were counted and cocultured with uninfected cells as in the previous
assays.

For all fusion assays, after 5 hr or 20 hr of cocultivation, the level of
25 cell fusion induced by the untreated recombinant or virus-infected cells and
the extent of fusion inhibition by the test compounds was evaluated by
microscopic observation. Fusion activities were determined by counting the
nuclei in syncytia compared with the total nuclei.

Influenza virus plaque assay

30 Phthalocyanine stock solutions at concentrations of 5 mg/ml diluted
10-fold in medium DMEM without FCS were used; 5 µl of compounds were
mixed with 45 µl influenza virus (A/PR/8/34[H1N1]) at a concentration of

approximately 3×10^3 infectious particles, and left in the dark at room
temperature for 1 hr. For MDCK plaque assay, 50 µl of virus/compound
mixture was mixed with 450 µl medium, and 200 µl of this mixture was
added to wells with confluent MDCK cells (6-well plate). After 1 hr

5   incubation, the cells were washed and agar containing 2x Dulbecco's
medium, 2.5 µg/ml trypsin, was added. After two days, agar with neutral red
was added. After three days the activity was measured by comparison of the
number of plaques in wells infected with compound-treated virus to the
number in wells infected with untreated virus.

10   *Cytotoxicity test*

A trypan blue exclusion test was used (Strober, Trypan blue
exclusion test of cell viability, p. A.3.3-A.3.4, in J.E. Coligan and A.M.
Kruisbeek (ed.), Current protocols in immunology, Wiley-Greene, New
York, N.Y., 1994). Compounds at a concentration of 50 µg/ml in growth

15   medium were added to a 96-well plate with MAGI cells. After 72 hr cells
were detached by 0.25% trypsin - 0.05% versene solution and diluted 1:10 in
growth medium. To test cell viability 1 part of 0.4% trypan blue was mixed
with 9 parts of diluted cells, the mixture incubated about 2 min at room
temperature, and a drop of the trypan blue/cell mixture added to a

20   hemacytometer. Stained (nonviable) and unstained (viable) cells were
counted using a binocular microscope. The fraction of viable cells was
calculated as the number of unstained cells in the wells treated with
compound as a percentage of the number in control wells.

Results

25   *Inhibition of viral infection*

Four classes of compounds were studied: phthalocyanines with
positively charged substituents, phthalocyanines with carboxylate
substituents, porphyrazines, and sulfonated phthalocyanines (Figure 1). The
positively charged phthalocyanines PcCat(1) and PcCat(2) had very poor

30   anti-HIV activity; PcCat(1) did not block viral infection and PcCat(2)
promoted growth of the virus (185 +/- 5%). The phthalocyanines bearing

carboxylate substituents had moderate to low activity. The Al and Co chelates blocked 40 +/- 15 and 19 +/- 2% of viral infection, respectively.

In general, the sulfonated phthalocyanines were the most active of the classes studied. Figure 5 shows the activity of sulfonated phthalocyanines

5   against HIV-1 IIB, which was assayed under the following conditions: compounds at a concentration of 50 μg/ml were incubated with HIV-1 IIIB in the dark for 1 hr, diluted 10-fold and used to inoculate MAGI cells. After three days activity against HIV was measured by removal of the media, fixation and staining with X-gal. The activity against HIV was measured by

10 dividing the number of blue cells in wells infected with compound-treated virus by the number in wells infected with untreated virus. In Figure 5, data are plotted as the mean of three experiments, each replicated twice. Error bars represent standard deviations.

There is a strong correlation between high activity and the absence of

15 a ligand on the central metal. The most active compounds are the parent PcS (no metal, 94% blocking of HIV infection) and its copper [CuPcS(1), essentially no binding of axial ligands, 97% blocking of HIV infection] and nickel (NiPcS, weak binding of axial ligands, 93% blocking of HIV infection) chelates. The VO derivative, which has only a tightly bound

20 oxygen atom as an axial ligand, also displayed good activity (86% blocking of HIV infection). The Al, Co, Cr, Fe, Mn, Si and Zn chelates, all of which have axial ligands, had lower activity, blocking 40 – 80% of HIV infection.

The copper chelate was studied further because it had high activity. In addition, because the copper is paramagnetic, copper phthalocyanines are

25 not sensitive to light (essentially eliminating any possible photodynamic side effects). Four commercially available samples were studied. CuPcS(1), CuPcS(2), CuPcS(H+) and CuPcS(Li+) had activities of 97, 72, 94 and 85% virus inhibition, respectively. It appears that some components of these mixtures of sulfonated copper phthalocyanines are more active than others.

30   *Kinetics of inactivation*

To determine the kinetics of inactivation of viral infectivity, mixtures of HIV-1 IIIB with the test compounds were incubated. The residual

infectivity was assayed at various time intervals. Two very active [NiPcS and CuPcS(1)], one moderately active [ZnPcS(1)] and two relatively inactive chelates [Co(II)PcS and AlPcC] were studied. For all five compounds, the inactivation level observed at two minutes was constant over the time period

5   studied (60 minutes), as shown by Figure 6. This observation indicates that the interaction of phthalocyanines with HIV-1 IIIB is both rapid and independent of time after two minutes.

Effective concentration

To determine the effective concentration of some of the more active

10   compounds, virus samples were mixed with phthalocyanines at defined concentrations. As shown by Figure 7, the most effective concentration was the generally the highest concentration of 50 μg/ml. However, three of the six compounds studied [CuPcS(1), CuPcS(H+) and NiPcS] also exhibited approximately 50% activity at concentrations of < 6.25 μg/ml. CuPcS(H+)

15   had approximately 50% activity at 0.6 μg/ml. Thus, the most active compound had an EC50 of < 1 μg/ml.

Activity after removal of phthalocyanine

It may be desirable for a potential microbicide to be virucidal, e.g., for inhibition of viral replication to continue even after the concentration of

20   the compounds is substantially decreased. A filtration-dilution protocol was used to show that the virus, once treated, was still rendered non-infectious once the unbound compound had been removed. In this protocol, solutions of the virus and compound were filtered until only about 10% of the original volume remained. The solution that had not gone through the filter was

25   diluted to the original volume and the process repeated four times. Spectroscopic assays showed that four dilutions resulted in the original compound concentrations being reduced by 35- to 500-fold. Three compounds were evaluated, NiPcS, PcS and ZnPcS(1). ZnPcS(1) had moderate activity in the screening assay. Filtration-dilution resulted in more

30   than a two-fold recovery of infectivity. NiPcS and PcS were both very effective in blocking of HIV infection; after filtration dilution there was only minimal recovery of infectivity. Thus, PcS and NiPcS are truly

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virucidal; that is, virus inhibition continues even after free compound is removed.

Activity against other viruses

To investigate whether the compounds with high activity against HIV would inactivate other viruses, the studies were extended to HIV-1 89.6, SIVmac1A11 and influenza virus (A/PR/8/34[H1N1]). The most active compounds against HIV-1 IIIB: CuPcS(1), NiPcS and PcS, were selected. These three compounds inactivated HIV-1 89.6 to the extents of 73, 83 and 83%, respectively. It was observed that SIVmac1A11 was also sensitive to the compounds: 82%, 89%, and 59% activation, respectively. To determine the specificity of these results we used influenza virus (A/PR/8/34[H1N1]) which is an unrelated enveloped virus. Influenza virus was less sensitive to these compounds: CuPcS(1), NiPcS, and PcS inactivated 44, 37 and 46% of the infectivity, respectively.

Toxicity

To confirm that the decreased numbers of HIV-infected cells observed in the MAGI assay were not a result of the toxicity of the phthalocyanines, the toxicity of the more active phthalocyanines was assessed. Compounds at a concentration of 50 μg/ml in growth medium were added to MAGI cells. This concentration is ten-fold higher than that used when the compounds are applied to MAGI cells for virus assay. After 72 hr, a trypan blue assay was used to compare cell viability in cells treated with compounds to untreated cells. Cells treated with CuPcS(1), CuPcS(H+), NiPcS, and PcS, retained about 88, 65, 72 and 53% viability, respectively. These results indicate that the observed antiviral effects are unlikely to reflect toxicity of the test compounds.

Effect of phthalocyanines on interaction of gp120 with CD4

To investigate the site of action of the compounds, the effect of phthalocyanines on binding of gp120 to its primary receptor, CD4 (Dalgleish et al., Nature 312:763-767 (1984)) was investigated. CD4 binding results in a conformational change in gp120 (Sattentau and Moore, J. Exp. Med. 174, 407-415 (1991)) that enables it to interact with a coreceptor, generally either

CCR5 or CXCR4 (Lapham, et al., Science 274:602-605 (1996)). A gp120-CD4 binding assay was used as described in Materials and Methods. The CuPcS(1), CuPcS(H+), NiPcS, and VOPcS all were very effective in blocking HIV infection and also showed substantial (or complete) inhibition

5 of gp120-CD4 binding. However, PcS, which also is effective in blocking HIV infection (94%), did not block binding of gp120 to CD4 effectively. ZnPcS(1) had good activity against HIV (77% blocking of HIV infection) but showed almost no inhibition of binding of gp120 to CD4. AlPcC had moderate activity against HIV (40% blocking of HIV infection) but

10 completely inhibited binding of gp120 to CD4. Thus, there seems to be no correlation of anti-HIV activity and inhibition of gp120-CD4 binding in this series. More than one mechanism of blocking of HIV infection is apparently operative.

Inhibition of HIV-induced cell fusion by phthalocyanines

15   One possible mechanism by which phthalocyanines might block viral infection is inhibition of the fusion activity of the viral Env proteins, which is required for viral entry. This was investigated by assaying cell fusion activity. About 2.5 x 103 Hep2 cells infected with VVenv1, the vaccinia recombinant expressing the HIV-1 IIIB Env protein, were added to

20 3T3CD4CXCR4 cells in a 96-well plate in the presence (1) or absence (2) of NiPcS and incubated for 5 hours for syncytium analysis; samples were fixed, and photographed under a phase-contrast microscope. The results are shown in Table 1.

Three different expression systems for the Env proteins were

25 compared. These systems differ in their expression of other encoded proteins.

The first studies were done with a recombinant vaccinia expression system, which is able to express high levels of Env. Two recombinants were used: one expressing the HIV-1 IIIB Env which has tropism for the X4

30 coreceptor (VVenv1), and the second expressing the Env of HIV-1 89.6, a primary viral isolate with dual tropism for both X4 and R5 coreceptors (VV89.6 envt). Essentially complete inhibition of HIV-induced cell fusion was observed with the parent, nickel, manganese and vanadyl sulfonated

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phthalocyanines (PcS, NiPcS, MnPcS and VOPcS), and all forms of the copper phthalocyanines [CuPcS(1), CuPcS(2), CuPcS(H+), and CuPcS(Li+)]. With the exception of the manganese chelate, these phthalocyanines bind axial ligands either not at all (parent, VO) or weakly,

5   leading to planar structures. In contrast, phthalocyanines bearing metals expected to bind axial ligands were not very effective at inhibiting fusion. All of AlPcC, AlPcS, Co(II)PcS, Co(III)PcS, CrPcS, ZnPcS(1) and ZnPcS(2) had at least 30% of nuclei in syncytia at 8 hr in one of the tests. Thus, inhibition of fusion is apparently most easily achieved with planar

10   phthalocyanines [i.e., those with no, or small (VO), axial ligands]. MnPcS is an exception; perhaps other mechanisms are important for this chelate, which is not considered further. A correlation between inhibition of fusion and blocking of viral infection was also observed. All the phthalocyanines that showed essentially complete inhibition of HIV-induced cell fusion, with the

15   exception of CuPcS(2), blocked more than 80% of viral infection. In contrast, the chelates that did not inhibit fusion significantly blocked less than 80% of viral infection and most blocked less than 60%.

TABLE 1. Inhibition of Env-induced cell fusion by phthalocyanines

Compound/ Constructs	Presence of nuclei in syncytia at indicated time(s) for construct:			
	pIIIenv X4, 8h/21h	VVenv1 X4, 8h/21h	VV89.6 envt X4, 8h/21h	VV89.6 envt R5, 8h/21h
Control	4+/4+	4+/4+	4+/4+	4+/4+
AIPcC	2+/4+	4+/4+	4+/4+	4+/4+
AIPcS(1)	3+/4+	3+/4+	4+/4+	4+/4+
Co(II)PcS	-/3+	-/3+	4+/4+	3+/4+
Co(III)PcS	-/-	-/-	-/4+ ·	3+/4+
CrPcS	-/-	-/3+	-/4+	2+/4+
CuPcS(1)	-/-	-/-	-/-	-/-
CuPcS(2)	-/-	-/-	-/-	-/-
CuPcS(H+)	-/-	-/-	-/-	-/-
CuPcS(Li+)	-/-	-/-	-/-	-/-
MnPcS	-	-/-	-/-	-/-
NiPcS	-	-/-	-/-	-/-
PcS	-/+1	-/-	-/-	-/-
SiPcS	2+/4+	4+/4+	4+/4+	4+/4+
VOPcS	-	-/-	-/-	-/-
ZnPcS(1)	-/1+	-/-	-/2+	1+/4+
ZnPcS(2)	2+/4+	2+/4+	3+/4+	3+/4+

Fusion activities were demonstrated by comparing the nuclei in syncytia to the total nuclei.
4+, more than 50% of nuclei are in syncytia; 3+, 30 to 50% of nuclei are in syncytia; 2+, 30
5   to 10% of nuclei are in syncytia; + less than 10% of nuclei are in syncytia; -, no syncytia
were observed.

In a second set of experiments, pIIIenv, a plasmid expression system

that is able to express Env proteins in the absence of other HIV proteins or

10   vaccinia proteins was used. As above, an essentially complete inhibition of

HIV-induced cell fusion with PcS, NiPcS, MnPcS, VOPcS, and all forms of

the copper phthalocyanines [CuPcS(1), CuPcS(2), CuPcS(H+), and

CuPcS(Li+)] was observed. PcS did not completely block fusion in the

pIIIenv recombinant (after 21 hr incubation, 10% of the cells were fused),

15   though it did inhibit fusion in the recombinant vaccinia expression system.

PcS also blocks gp120/CD4 binding by only about 35%.

For comparison, cells persistently infected with HIV-1 IIIB or HIV-1 89.6 were used for cocultivation with uninfected target cells in the presence or absence of test compounds. The fusion activity observed in this assay was comparable with that found using plasmids expressing Env, and lower than

5   observed with Env expressed by vaccinia virus. The results of fusion inhibition by the compounds tested correlated well with results observed using both other expression systems.

These results demonstrate that the compounds with high or intermediate levels of anti-HIV activity are able to effectively inhibit the

10   membrane fusion activity of the viral Env proteins, a biological function that is important for viral entry as well as the induction of viral cytopathic effects.

Discussion

The central goal of this study was to identify compounds with high activity in blocking HIV infection, which could be useful as topical

15   formulations to provide a defense against infection by sexually transmitted virus. The vaginal and gastrointestinal surfaces play a major role in the pathogenesis of infection by HIV-1 as potential routes for viral entry. A MAGI assay which is based on usage of an epithelial cell line was used as an assay for anti-HIV activity of test compounds. HeLa cells stably transfected

20   with human CD4 (HeLa-CD4 cells) are permissive for T cell line-adapted X4 or dual usage R5X4 viruses, because they express the coreceptor CXCR4. All the anionic phthalocyanines studied were active with various degrees of efficacy against HIV-1 IIIB. Overall, the most promising compounds were CuPcS(1), CuPcS(H+), and NiPcS. These compounds were also able to

25   inhibit infection by dual tropic HIV-1 89.6 as well as SIVmac1A11 viruses.

To determine the specificity of the phthalocyanine-virus interaction, the activity of the compounds against HIV-IIIB were compared with that against the influenza virus. It was observed that influenza virus was relatively insensitive to compounds with good activity against HIV. These

30   results suggest that the virucidal effect of these compounds is a result of their interaction with specific viral components, rather than a general disruptive effect on enveloped virus structure.

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It was also observed that compounds with anti-HIV activity were able to inhibit the cell fusion activity of the HIV Env protein. To exclude the possibility that such an inhibitory effect could be due to an indirect effect on surface expression of the Env protein, we demonstrated that cell fusion
5   induced by recombinant vectors in the absence of any other HIV protein was also sensitive to inhibition by the compounds. These results provide strong evidence that the phthalocyanines are able to inhibit an important function of the Env protein that is needed for viral entry. Structure activity correlations indicate that planar or nearly-planar phthalocyanines are more effective than
10   phthalocyanines with axial ligands both in inhibition of fusion and blocking of viral infection.

The sulfonated phthalocyanines studied are small herein polyanionic molecules. They inhibit viral binding and fusion/entry into susceptible cells. In this they are similar to other polyanionic species, including the sulfated
15   polymers (De Clercq, 2002b). The interaction of the phthalocyanines with HIV appears to be very rapid. For PcS and NiPcS, removal of free compound did not result in significant recovery of infectivity, indicating that these are effective virucidal agents. Virucidal activity is desirable, but is presumably not necessary, in that the compounds when used as microbicides
20   will continue to be present at sites of transmission during exposure to virus *in vivo*. Sulfonated phthalocyanines therefore are a promising class of compounds for further development as microbicides to prevent HIV transmission. Specific structural features, particularly the central metal and perhaps the extent and placement of the sulfonic acid groups, also play a role
25   in determining the activity of these compounds.

We claim:

1. A method for decreasing a viral infection in a human comprising administering to a mucosal surface a composition containing an amount of a neutral or negatively-charged compound, or a metallocomplex of the compound, or a pharmaceutically acceptable salt of the compound or metallocomplex, or combination thereof, to a human in an amount effective to decrease a viral infection or sexually transmitted disease (STD) , wherein the compound has one of the following structures:

Formula I   Formula II

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} taken independently or together can be hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, nitro, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, heteroarylthio, substituted heteroarylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfinyl, substituted sulfinyl, sulfonyl, substituted sulfonyl, sulfonic acid, substituted sulfonic acid, phosphonato, substituted phosphonato, phosphoramide, polyaryl, substituted polyaryl, C1-C20 cyclic, substituted C1-C20 cyclic, heterocyclic, substituted heterocyclic, aminoacid, peptide, or polypeptide group,

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wherein the metallocomplex is formed of the compound with a metal selected from the group consisting of main group or transition metal atoms, and

wherein the compound, the metallocomplex, or the pharmaceutically salt of the compound or metallocomplex is in an effective amount to decrease the viral infection or virus proliferation or sexually transmitted disease.

2. The method of claim 1 wherein the compound is effective to decrease a viral infection or virus proliferation caused by a virus selected from the group consisting of HIV viruses, hepatitis B and C viruses, and papilloma viruses.

3. The method of claim 2 wherein the compound is effective to decrease a viral infection or virus proliferation caused by an HIV virus.

4. The method of claim 3 wherein the compound is a sulfonated copper phthalocyanine.

5. The method of claim 1 wherein the metallocomplex is formed of a metal selected from the group consisting of gallium (Ga), aluminum (Al), cadmium (Cd), ruthenium (Ru), rhodium (Rh), platinum (Pt), palladium (Pd), osmium (Os), iridium (Ir), iron (Fe), cobalt (Co), zinc (Zn), molybdenum (Mo), titanium (Ti), manganese (Mn), chromium (Cr), nickel (Ni), magnesium (Mg), copper (Cu), indium (In), vanadium (V), silver (Ag), gold (Au), and tin (Sn).

6. The method of claim 5 wherein the metal is selected from the group consisting of Cu, Fe, Mn, Ni, and V.

7. The method of claim 1 wherein one or more of the groups R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} of the compound is either a sulfonate or has a sulfonate as a substituent.

8. The method of claim 1 wherein the compound is present in more than one form.

9. The method of claim 1 wherein the pharmaceutically acceptable salt is a salt with a cation selected from the group consisting of H+, Na+, Li+, K+, Ca++, Mg++, protonated amines and heterocyclic amines, and quaternary ammonium ions with substituents selected from the group

consisting of alkyl and aryl groups which optionally have heteroatoms in the structure.

10. The method of claim 1 wherein the compound is protected against rapid elimination from the body.

11. The method of claim 1 further comprising a pharmaceutically acceptable carrier, wherein the carrier is selected from the group consisting of ointments, creams, gels, liposomes, nanoparticulates, and microspheres and lotions.

12. The method of claim 1 comprising formulating the compound with a pharmaceutically acceptable carrier selected from the group consisting of troches, suppositories, and vaginal rings.

13. The method of claim 1 further comprising encapsulating the compound in a biodegradable implant.

14. The method of claim 1 further comprising providing a liposomal suspension or hydrogel for delivery of the compound.

15. The method of claim 1 further comprising mixing with the compound with compounds selected from the group consisting of water for injection, saline, oils, polyethylene glycols, glycerine, propylene glycol, antibacterial agents, antioxidants, chelating agents, buffers, and agents for adjusting tonicity.

16. The method of claim 1 further comprising co-administering at least one compound selected from the group consisting of antibiotics, virucidals, antivirals, antifungals, contraceptives, immunosuppressants and immunostimulants.

17. The method of claim 1 further comprising co-administering at least one compound selected from the group consisting of Carraguard, Buffergel, antibodies, defensins, cyclodextrins, and polyethylene hexamethylene biguanide.

18. The method of claim 1 further comprising co-administering at least one antiviral agent selected from the group consisting of HPA-23, interferons, ribavirin, phosphonoformate, ansamycin, suramin, imuthiol, penicillamine, carbovir, 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (DDC), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxyadenosine

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(DDA), 3'-azido-2',3'-dideoxyuridine (CS-87), 2',3'-dideoxy-2',3'-didehydrocytidine (D4C), 3'-deoxy-2',3'-didehydrothymidine (D4T) and 3'-azido-5-ethyl-2',3'-dideoxyuridine (CS-85).
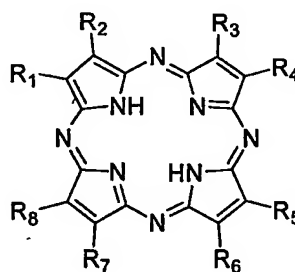
19.   The method of claim 1 wherein the composition is administered topically or to a mucosal surface.

20.   The method of claim 19 wherein the composition is administered to the rectum or female genital tract.

21.   A composition for mucosal administration containing a neutral or negatively charged compound, a metallocomplex of the compound, a pharmaceutically acceptable salt of the compound or the metallocomplex, or a mixture thereof, wherein the compound has the one of the following structure:

Formula I   Formula II

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} taken independently or together can be hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, nitro, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, heteroarylthio, substituted heteroarylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfinyl, substituted sulfinyl, sulfonyl, substituted sulfonyl, sulfonic acid, substituted sulfonic acid, phosphonato, substituted

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phosphonato, phosphoramide, polyaryl, substituted polyaryl, C1-C20 cyclic, substituted C1-C20 cyclic, heterocyclic, substituted heterocyclic, aminoacid, peptide, or polypeptide group,

wherein the metallocomplex is formed of the compound with a metal compound wherein the metal compound has a metal selected from the group consisting of main group or transition metal atoms,

wherein the compound or metallocomplex of the compound is not one selected from the group consisting of copper phthalocyanine tetrasulfonic acid tetrasodium salt; nickel phthalocyanine tetrasulfonic acid, copper phthalocyanine 3,4',4",4'''-tetrasulfonic acid, copper phthalocyanine, copper-4,4',4",4'''-tetraaza29H, 31H-phthalocyanine, cobalt phthalocyanine, reactive blue 15, and silicon phthalocyanine dichloride, and

a pharmaceutically acceptable carrier for administration to a mucosal surface.

22.   The composition of claim 21 wherein the metal is selected from the group consisting of gallium (Ga), aluminum (Al), cadmium (Cd), ruthenium (Ru), rhodium (Rh), platinum (Pt), palladium (Pd), osmium (Os), iridium (Ir), iron (Fe), cobalt (Co), zinc (Zn), molybdenum (Mo), titanium (Ti), manganese (Mn), chromium (Cr), nickel (Ni), magnesium (Mg), copper (Cu), indium (In), vanadium (V) or vanadium oxide (VO), silver (Ag), gold (Au) and tin (Sn), and

wherein the metal atom optionally has one or more negatively charged or neutral ligands.

23. The composition of claim 21 wherein the carrier is selected from the group consisting of ointments, creams, gels, lotions, liposomes, nanoparticulates, microspheres, and controlled release formulations.

24. The composition of claim 21 wherein the carrier is selected from the group consisting of troches, suppositories, and vaginal rings.

25. The composition of claim 21 suitable for administration to the female genital tract.

26. The composition of claim 21 suitable for rectal administration.

27. The composition of claim 21 wherein the compound is effective for decreasing infection or virus proliferation of a sexually transmitted disease.

28. The composition of claim 21 wherein the compound is effective for decreasing infection by an HIV or decreasing proliferation of the HIV virus.

29. The composition of claim 28 wherein one or more of the groups R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} of the compound is either a sulfonate or has a sulfonate as a substituent and mixtures of such structures.

30. The composition of claim 21 wherein the metal is selected from the group consisting of Cu, Fe, Ni, Mn, and V.

31. The composition of claim 21 wherein the pharmaceutically acceptable salt is a salt with a cation selected from the group consisting of H+, Na+, Li+, K+, Ca++, Mg++, and protonated amines and heterocyclic amines, and quaternary ammonium ions with substituents selected from the group consisting of alkyl and aryl groups which optionally have heteroatoms in the structure.

32. The composition of claim 21 further comprising at least one other agent selected from the group consisting of antibiotics, virucidals, antivirals, antifungals, contraceptives, immunosuppressants, and immunostimulants.

33. The composition of claim 32 wherein the agent is selected from the group consisting of Carraguard, Buffergel, antibodies, defensins, cyclodextrins, and polyethylene hexamethylene biguanide.

34. The composition of claim 21 wherein the virucidal or antiviral agent is selected from the group consisting of HPA-23, interferons, ribavirin, phosphonoformate, ansamycin, suramin, imuthiol, penicillamine, carbovir, 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (DDC), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxyadenosine (DDA), 3'-azido-2',3'-dideoxyuridine (CS-87), 2',3'-dideoxy-2',3'-didehydrocytidine (D4C), 3'-deoxy-2',3'-didehydrothymidine (D4T) and 3'-azido-5-ethyl-2',3'-dideoxyuridine (CS-85).
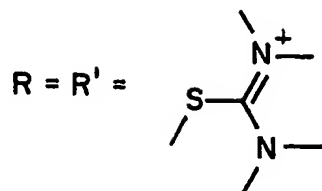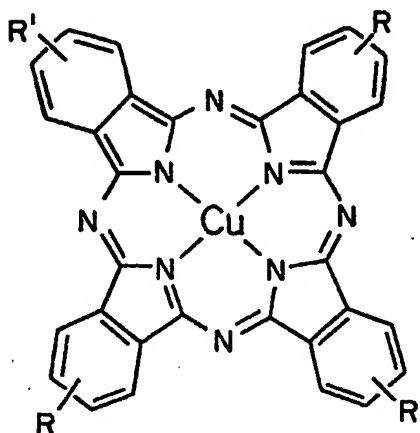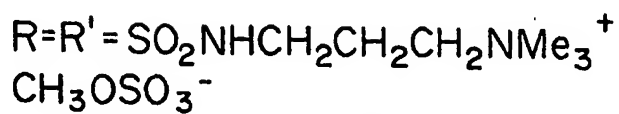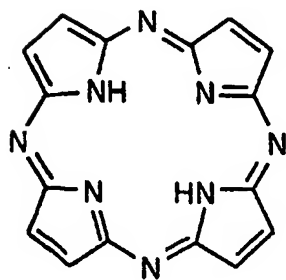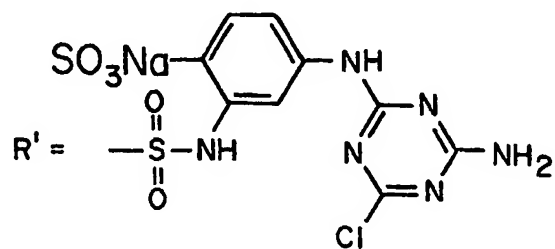
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35. The composition of claim 21 wherein the pharmaceutically acceptable salt is a salt with a cation selected from the group consisting of H+, Na+, Li+, K+, Ca++, Mg++, protonated amines and heterocyclic amines, and quaternary ammonium ions with substituents selected from the group consisting of alkyl and aryl groups which optionally have heteroatoms in the structure.

PcS

PcC

$R = R' =$ Alcian Blue

$R = R' = SO_2NHCH_2CH_2CH_2NMe_3^+$
$CH_3OSO_3^-$

Alec Blue

$R = SO_3Na$

$R' =$
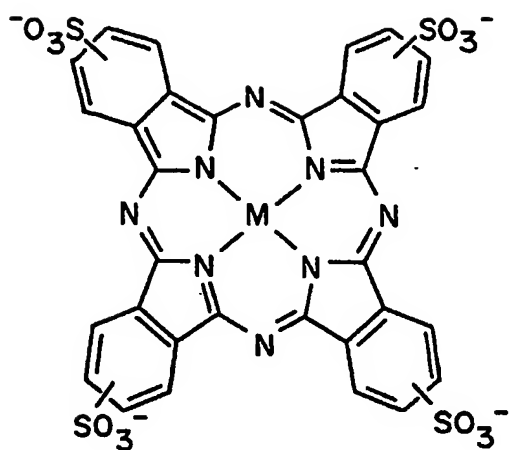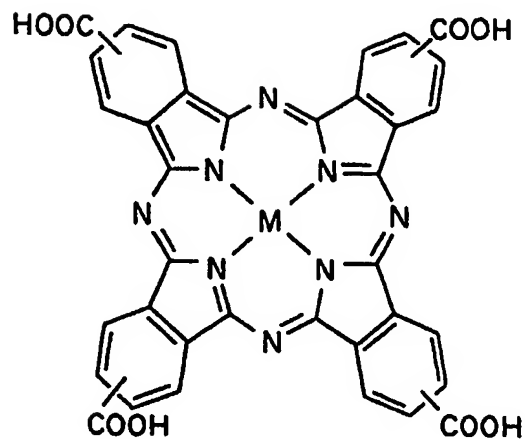
porphyrazine

Reactive Blue 15

FIG. 1

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FIG. 2

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FIG. 3

FIG. 4

FIG. 5

FIG. 6

- ◆ AlPcC
- ✕ NiPcS
- ✳ ZnPcS
- ■ Co(II) PcS
- ▲ CuPcS(1)

FIG. 7

- ⊞ 0.6µg/ml
- ▨ 6.25 µg/ml
- ☐ 12.5µg/ml
- ▨ 25µg/ml
- ⊞ 50µg/ml

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FIG. 8